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Isolation and Chemical Characterization of Water-soluble Arabinoxylans in Rye Grain

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ABSTRACT

Rye grain (cv. Kungs II) was ground and refluxed in 90% aqueous ethanol in order to inactivate endogenous enzymes and remove low molecular weight sugars. The residue was extracted with water at 40°C and a crude arabinoxylan isolated from the extract by precipitations with ammonium sulphate and 67% aqueous ethanol. The crude arabinoxylan constituted 0·74% of the whole-grain and contained 72% arabinoxylans.

The crude arabinoxylan was fractionated on a DEAE-cellulose column. The main fraction was eluted with water and contained arabinose and xylose residues in a ratio of 1:2·1, together with traces of other components. The quantitatively second most important fraction was eluted with weak borate and contained arabinose and xylose residues in a ratio of 1:1·8, together with significant amounts of glucose residues and non-carbohydrate components.

Methylation and ^{1}H- and ^{13}C-NMR analysis revealed that the water-eluted arabinoxylan contained a main chain of 4-linked β-D-xylopyranosyl residues of which about 50% were substituted at position three with terminal α-L-arabinofuranosyl residues. About 2% of the xylose residues were double-substituted at positions two and three by terminal α-L-arabinofuranosyl residues. The same structural units were found in the fraction eluted with weak borate but the branched and double-branched units were more abundant.

INTRODUCTION

Rye is a widely grown cereal and the grain is used both in bread and other products for human consumption and in animal feeds. Compared

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to other cereals, rye contains high amounts of arabinoxylans (pentosans) of which about 30% are soluble in hot buffer, giving rise to highly viscous solutions (Podrazký, 1964; Pettersson & Åman, 1987). It has also been shown that the molecular weights of soluble arabinoxylans in rye flour are about 2–3 times greater than those isolated from wheat flour (Meuser & Suckow, 1986).

Rye grain has a nutrient content which is similar to that of wheat (Miller, 1958) but the use of rye in animal feeds is restricted because of the presence of various toxic or antinutritional factors which reduce its nutritive value, particularly for monogastric animals. The arabinoxylans have recently been shown to be the main antinutritional factor (Antoniou *et al.*, 1981), and considerable improvements in growth rate, feed intake and feed conversion efficiency have been obtained when rye-based chicken feeds were supplemented with an enzyme preparation containing arabinoxylanase activities (Pettersson & Åman, 1988). The influence of pentosans on the milling behaviour of grain (Moss & Stenvert, 1971), the quality of flour (Tao & Pomeranz, 1967), the rheological properties of dough (Mod *et al.*, 1981) and the bread quality (Meuser & Suckow, 1986) have been reported. In human nutrition, positive effects of dietary fibres in rye on sugar and lipid metabolism have been found (Hagander *et al.*, 1987).

In spite of the technical and nutritional importance of pentosans in rye only a few modern studies on the structure of arabinoxylans in rye grain have been reported (Fincher & Stone, 1986; Hromádková *et al.*, 1987). In the present investigation water-soluble arabinoxylans from whole-grain rye were fractionated on a DEAE-cellulose column and the structural features of the main fractions investigated.

MATERIALS AND METHODS

Material

Winter rye (cv. Kungs II) was harvested during 1986 in the south of Sweden near Landskrona. The grain was dried and stored as whole kernels.

General analyses

Prior to analysis, a representative sample (100 g) of the grain (94% dry matter) was ground to pass a 0·5 mm screen. All analyses were carried out in duplicate and are reported on a dry matter basis. The dry matter

content was determined by drying at 105°C for 16 h. Free sugars, including glucose, fructose, sucrose and fructans, were extracted with 0·05 M sodium acetate buffer (pH 5·0) at 65°C and determined enzymatically (Larsson & Bengtsson, 1983). Crude protein (Nx6·25) and ash were determined according to standard methods (AOAC, 1980). Crude fat was extracted with diethyl ether in an Tecator Soxtec System HT after acid hydrolysis (Anon., 1971). Starch (Åman & Hesselman, 1984) and mixed-linked (1→3), (1→4)-β-D-glucan (Åman & Graham, 1987), in the following referred to as β-glucan, were determined by enzymatic methods. Total dietary fibre, including non-starch polysaccharides and Klason lignin, was determined according to Theander and Westerlund (1986).

Isolation of water-soluble arabinoxylans

Rye grain was ground in a Wiley mill to pass a 1·5 mm screen. The meal (300 g) was suspended in 90% aqueous ethanol (600 ml) and refluxed in a boiling water-bath for 10 min. The insoluble residue was isolated and washed (600 ml of the aqueous ethanol) by centrifugation (3000 g, 10 min). This residue was suspended in water (500 ml) and treated in a water-bath at 40°C for 90 min. In the middle of this treatment the slurry was homogenized in a Sorvall Ultra-Turrax for 2 min. Soluble components (A) were isolated by centrifugation as above, and soluble arabinoxylans precipitated by the addition of 200 g ammonium sulfate (Preece & Hobkirk, 1953; McCleary, 1986). The precipitate was left in a refrigerator overnight and thereafter isolated by centrifugation as above (B). The precipitate was suspended in hot water (500 ml, 80°C) using the Ultra-Turrax and reprecipitated by the addition of 95% ethanol (1200 ml). The precipitate was left in a refrigerator overnight, isolated and washed (500 ml 80% aqueous ethanol) by centrifugation as above. The isolated precipitate was again suspended in hot water (600 ml, 80°C) using the Ultra-Turrax, dialysed against distilled water for three days and freeze-dried to give a crude arabinoxylan (C; yield 0·74% of dry meal).

Fractionation on DEAE-cellulose

The crude arabinoxylan (500 mg) was suspended in 35 ml of water using the Ultra-Turrax. After centrifugation (3000 g, 10 min) the supernatant was applied on a DEAE-cellulose (type DEAE-SS, Serva Feinbiochemica, Heidelberg) column (2·6 × 50 cm) which was regenerated and eluted with water (Fraction I), 0·0025 M (Fraction II) and 0·025 M sodium borate (Fraction III), 0·4 M sodium hydroxide (Fraction IV) and saturated

sodium borate (Fraction V) as described by Neukom *et al.* (1960). Fractions (10 ml) were collected and their carbohydrate content determined using the phenol-sulphuric acid method (Dubois *et al.*, 1956). The carbohydrate-containing fractions from each eluent were pooled, dialysed and freeze-dried. Optical rotation of the main fractions were measured with a Perkin Elmer 141 polarimeter.

Sugar analysis

The isolated polysaccharide fractions (2 mg) were hydrolyzed in 1 M trifluoroacetic acid (121°C, 90 min). The formed sugars were reduced, acetylated and analyzed by GLC on a CP-Sil 88 capillary column (Theander & Westerlund, 1986).

Methylation analysis

Isolated polysaccharide fractions (1 mg) were dried in a vacuum, methylated by a modified Hakamori method, hydrolyzed with trifluoroacetic acid, reduced and acetylated (Harris *et al.*, 1984).

The identities of the O-acetylated, O-methylated alditols were established by their GLC retention times on a DB-1 capillary column (Oakley *et al.*, 1985) and by their e.i. mass spectra (Björndal *et al.*, 1970) using a Finnegan 4021 mass spectrometer at 70 eV and an Incos 2000 data system.

NMR spectroscopy

^{1}H-NMR spectra (400 MHz) were recorded at 85°C on a Varian VXR 400 instrument. About 2000 pulses were collected, pulse repetition time was 3·75 s and r.f. pulse angle 45°. The dried polysaccharide fractions (1–4 mg) were dissolved in D_2O (0·7 ml) and sodium 3-trimethylsilyltetradeuteriopropionate was used as reference.

^{13}C-NMR spectra (101 MHz) were run at 85°C on the same instrument. About 90 000 pulses were collected, pulse repetition time was 2·50 s and r.f. pulse angle 70°. The dried polysaccharide fractions (50–100 mg) were dissolved in D_2O (3 ml) and internal dioxan was used as a reference (67·4 ppm).

RESULTS AND DISCUSSION

Starch, dietary fibre and crude protein were the main components of the whole-grain rye and together constituted more than 90% of the dry

matter (Table 1). Of the dietary fibre constituents arabinoxylans dominated with significant amounts of cellulose, Klason lignin and β-glucan.

Whole-grain rye was milled and refluxed with 90% ethanol in order to remove low-molecular weight compounds and inactivate endogenous enzymes. The residue was extracted with water, giving an extract (A) rich in polysaccharides containing arabinose, xylose and glucose residues (Table 2). Using ammonium sulfate precipitation, a fraction (B) with reduced content of glucose but also of arabinose residues was isolated. This fraction was dissolved in water and precipitated with 67% aqueous ethanol, giving the crude arabinoxylan (C) with a low content of glucose residues. The low content of glucose residues showed that the starch content was low in the crude arabinoxylan and consequently no amylase treatment was applied.

The crude arabinoxylan was fractionated on a DEAE–cellulose column. The main fraction, containing about 43% of the applied material, was not bound to the column, eluted with water (I) and contained arabinose and xylose residues in a ratio of 1:2·1, together with small amounts of glucose residues and only traces of other components (Table 3). The second most important fraction, containing about 12% of the applied material, was eluted with the weak sodium borate buffer (II). This fraction contained arabinose and xylose residues in a ratio of 1:1·8

TABLE 1
Chemical Composition of Whole-Grain Rye

Component	*% Dry matter*
Glucose	0·4
Fructose	0·1
Sucrose	2·9
Fructans	2·7
Starch	64·6
Crude protein	9·5
Crude fat	2·4
Ash	1·9
Dietary fibre	16·5
β-Glucans	2·3
Arabinoxylans[a]	7·6
Cellulose[b]	2·6
Klason lignin	3·0

[a]Arabinose and xylose residues of non-starch polysaccharides.
[b]Glucose residues of non-starch polysaccharides minus β-glucans.

TABLE 2
Yield of Dry Matter and Arabinose, Xylose and Glucose Residues, Given as Per Cent of Dry Meal, and Ratios of these Sugar Residues During Isolation of the Crude Arabinoxylan

Fraction[a]	*DM*	*Ara*	*Xyl*	*Glc*	*Ara/Xyl*	*Ara+Xyl/Glc*
Water extract (A)	—[b]	0·42	0·69	0·96	0·61	1·16
Ammonium sulphate precipitate (B)	—	0·16	0·33	0·12	0·48	4·08
Crude arabinoxylan (C)	0·74	0·17	0·36	0·03	0·47	17·67

[a]See Materials and Methods for more details regarding fractions.
[b]— Not determined.

TABLE 3
Glycosyl Composition[a] of the Crude Arabinoxylan and Fractions (I–V)[b] from the DEAE-Cellulose Column

Glycosyl residue	*Crude arabinoxylan (C)*	*I*	*II*	*III*	*IV*	*V*
Arabinose	23·0	30·8	27·3	7·3	10·4	9·7
Xylose	48·6	64·5	50·0	14·1	16·6	9·7
Galactose	trace	—	—	—	trace	trace
Glucose	4·1	3·3	10·5	7·8	26·8	29·0

[a]Given as % of dry fraction.
[b]For details see Materials and Methods.

together with significant amounts of glucose residues and non-carbohydrate components. The fraction eluted with 0·4 M sodium hydroxide (IV) was also significant and contained about 10% of the applied material. Glucose and non-carbohydrate components were dominating in this fraction and the ratio of arabinose and xylose residues was 1:1·6.

The main arabinoxylan-containing fractions (I and II) were further studied. Both fractions had highly negative optical rotations, $[\alpha]_D^{25}$ −94·5° and −92·9° respectively (c = 1·0%, H_2O) and were very viscous.

Linkage analysis (Table 4) revealed that fractions I and II were composed of terminal arabinofuranosyl residues and 4-, 3,4- and 2,3,4-linked xylopyranosyl residues. In fraction II small amounts of 4-linked glucopyranosyl residues were also detected. The product composition showed that fraction II contained more branched and double-branched

TABLE 4
Glycosyl Linkage Composition of Fractions (I and II) from the DEAE-Cellulose Column

Residue	*Positions of O-methyl groups*	*T*[a]	*Product composition (mol %)*		*Structural unit deduced*
			I	*II*	
Ara	2,3,5	390	28	28	Ara(f)→
Xyl	2,3	538	36	30	→4- Xyl(p)→
Xyl	2	638	34	37	→3,4- Xyl(p)→
Glu	2,3,6	719	—	1	→4- Glc(p)→
Xyl	—	728	2	4	→2,3,4-Xyl(p)→

[a]Retention time (×1000) of the corresponding alditol acetate relative to that of *myo*-inositolhexa-acetate on a DB-1 capillary column (2 min at 150°C, 150–225°C at 3°C/min, and 225°C until the last peak eluted).

xylose residues compared to fraction I. Methylated/acetylated derivatives of terminal arabinofuranosyl residues are volatile and easily lost during the analysis, which may explain the low molecular percent of this component compared to the branched xylose residues. It is generally accepted that the configuration of arabinose residues is L and of xylose residues is D in arabinoxylans of cereals (Aspinall & Sturgeon, 1957).

^{1}H-NMR analysis of fraction I and II (Fig. 1(a) and (b)) revealed signals for anomeric protons of terminal α-D-arabinofuranosyl residues at 5·2, 5·3 and 5·4 ppm and of β-D-xylopyranosyl residues at 4·4–4·7 ppm (Capon & Thacker, 1964; De Silva *et al.*, 1986). The signal at 5·4 ppm was assigned to the terminal arabinofuranosyl residues linked to 0–3 of the branched xylopyranosyl residues and the signals at 5·2 and 5·3 ppm to the terminal arabinofuranosyl residues linked to 0–2 and 0–3 of the double-branched xylopyranosyl residues by comparing their relative intensities. The equal size of the signals at 5·2 and 5·3 ppm was confirmed in several different arabinoxylan fractions. The complexity of the main chain of xylopyranosyl residues is indicated by the broad peak around 4·5 ppm and the small signal at 4·67 ppm was assigned to the double-branched xylopyranosyl residues. The other protons of the sugar residues are found at 3·2–4·3 ppm, which may be identified by comparing with reference substances. Integrations of the signals in the anomeric region showed a ratio of 1:2·1 in fraction I (Fig. 1(a)) and 1:1·6 in

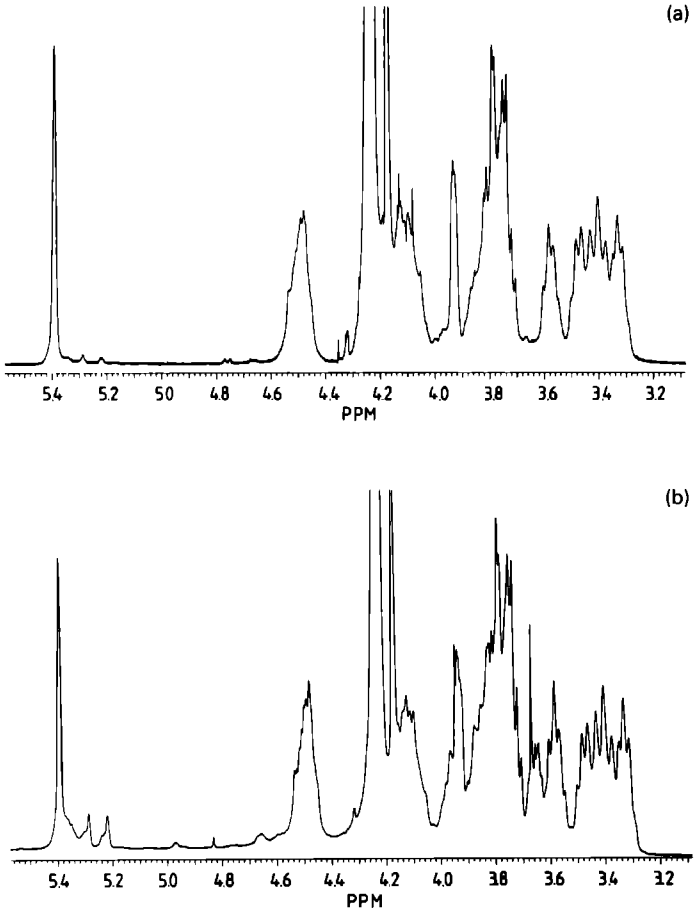

Fig. 1. ^{1}H-NMR spectra of fraction I (a) and II (b) from the DEAE-cellulose column. The chemical shifts were assigned relative to Me_3 SiCD Co_2Na.

fraction II (Fig. 1(b)) for arabinose to xylose residues. The ratios are in agreement with the sugar analysis. The higher content of branched and double-branched residues in fraction II was also evident.

A ^{13}C-NMR spectrum of fraction I is shown in Fig. 2. The narrow resonances at 62·4, 78·1, 81·8, 85·6 and 108·5 ppm were assigned to C-5, C-3, C-2, C-4 and C-1 of the terminal arabinofuranosyl residues by comparing with data from literature (Hromádková *et al.*, 1987). The resonances around 63·9, 73·9, 74·6, 77·3 and 102·5 ppm were assigned to C-5, C-2, C-3, C-4 and C-1 of the unbranched xylose residues by comparing with published data (Bock *et al.*, 1984) and results from an unbranched xylan isolated by alkaline extraction of red clover stems

(Nordkvist, E. & Åman, P., unpublished). Finally, resonances around 63·7, 73·6, 74·6, 78·5 and 102·0 ppm were assigned to C-5, C-2, C-4, C-3 and C-1 of the branched xylose residues by considering changes due to the 3-substitution and relative intensities of the signals. The multiple but well-defined appearance of the resonances from the xylose residues indicated an ordered and not too complex distribution of side chains along the main xylose chain. Fraction II gave similar ^{13}C-NMR spectra although the relative intensities of the main signals were changed and new signals of low intensities, probably from the double-branched units, could be detected.

Arabinoxylans from rye have been studied previously (Aspinall & Sturgeon, 1957; Aspinall *et al.*, 1960) and the results in the present paper confirm a structure with a high degree of branching. The main fraction, eluted with water, contained arabinose and xylose residues in a ratio of about 1:2. The terminal arabinofuranosyl residues were mainly linked to 0–3 of the backbone of (1→4)-linked xylopyranosyl residues but some were also substituted at 0–2 and 0–3 (Fincher & Stone, 1986). The same structural units were found in the fraction eluted with weak borate but the branched and double-branched units were more abundant.

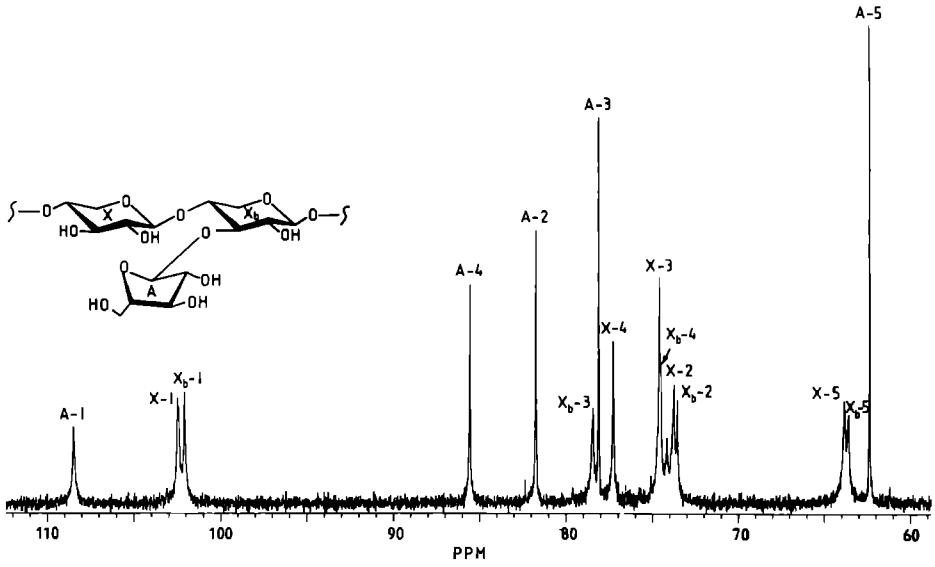

Fig. 2. ^{13}C-NMR spectrum of fraction I from the DEAE-cellulose column. The chemical shifts were assigned relative to internal dioxan.

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